MORPHOANATOMICAL STUDIES ON *Solanum nigrum* INFECTED WITH ROOT-KNOT NEMATODE, *Meloidogyne incognita*.

DISSERTATION

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in

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Dedicated

to my Everloving

Parents

and my

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Certificate

This is to certify that the work embodied in this dissertation entitled **"MORPHOANATOMICAL STUDIES ON *Solanum nigrum* INFECTED WITH ROOT-KNOT NEMATODE, *Meloidogyne incognita*"** is the bonafide work carried out by ***Miss Saba Aziz*** under my supervision and is suitable for submission for the M.Phil degree in Botany of Aligarh Muslim University, Aligarh.

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(Dr. Hisamuddin)

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Introduction

INTRODUCTION

Herbal medicines have been in use since time immemorial. They are the vital components of our natural and medical heritage; and there is immense satisfaction to be had in growing, harvesting and processing herbs for home use. Herbs have been prized for their pain relieving and healing abilities and, today we still rely on the curative properties of plants in about 75% of our medicines. Over the centuries, societies around the world have developed their own traditions to make sense of medicinal plants and their uses. Some of these traditions and medicinal practices may seem strange and magical; others appear rational and sensible, but all of them attempt to overcome illness and suffering and to enhance quality of life.

One of the most common medicinal weed plant is *Solanum nigrum*. It belongs to the family Solanaceae and is commonly called as "Black Nightshade" or "Common Nightshade". It is a very important medicinal plant and is used as source of herbal medicines. The plant is widely distributed throughout India and Pakistan.

Solanum nigrum is an erect, leafy, annual prickleless plant. The leaves are petiolate, thin, smooth and ovate. The margins

are wavy or bluntly lobed with a few broad teeth. The inflorescence is cymose. Flowers are small, white, axillary or lateral, drooping with long pedicel. The fruits are berries which are round, about half an inch in diameter. The berries are green at first, red, yellow or black when ripe.

There are three different varieties of *S. nigrum* (n=12, 24 and 36). The diploid varieties constitute a complex assemblage. Each variety differs from the other in minor morphological characteristic. These varieties have not contributed directly to the evolution of the naturally occurring tetraploids. The studies have revealed that the tetraploids, closely resemble with *S. luteum* in their morphology, cytology and in chemical nature of fruit pigments indicating that the tetraploids might be a geographical race of *S. luteum*. The hexaploid forms occur mostly in temperate regions and very rarely in warmer regions (Chadha, 1972).

The use of *S. nirgum* as stock for tomatoes to counteract the heat in North India has been suggested. The herb has antiseptic and antidysenteric properties and is given internally for cardalgia and gripe. An infusion of the plant is used as an enema in infants having abdominal upsets. It is a household remedy for

anthrax pustules and is applied locally. The plant is also credited with emollient diuretic and laxative properties and its decoction is regarded as an antispasmodic and narcotic. Freshly prepared extract of the plant is effective in the treatment of cirrhosis of the liver, and also serves as an antidote to opium poisoning. An alcoholic extract of leaves is active against *Staphylococcus aureus and Escherichia coli.*

Infusions or decoctions of the plant, after transient stimulation, depress the central nervous system and the reflexes of the spinal cord. Small doses increase and large doses decrease cardiac activity and consequently reduction in blood pressure. Extracts of the plant affect the rate and amplitude of respiration. (Chadha, 1972).

Leaves are used in the treatment of scrofulous dyscrasias, and are said to produce diaphoresis when in overdose; they also cause nausea, purging and nervous disturbances. In China, leaves are applied to wounds and sores. The juice of fresh leaves is reported to produce dilatation of the pupil. In Philippines, the pounded leaves are rubbed on depigmented areas of the body for restoring the pigment. (Chadha, 1972).

The chemical components of the leaf are as follows (in 100g edible material): water 82.1g, proteins 5.9g, fats 1.0g, minerals 2.1g, carbohydrates 8.9g, calcium 410mg, phosphorus 70mg, and Iron 20.5mg. Leaf is a rich source of riboflavin. The values for various vitamins present in the leaf are (in 100gm): riboflavin 0.59mg, nicotinic acid 0.92mg and vitamin C 11.0 mg.

Higher values for vitamin C (20-40mg/100gm) have also been reported. The content of β-carotene is 0.74 mg/100 gm of leaf whereas α-carotene content is negligible. Citric acid is present to the extent of 0.5%. (Chadha, 1972).

The berries possess tonic, diuretic and cathartic properties and are useful in anasarca and heart diseases. These are the source of domestic remedies for fevers, diarrhoea, ulcers and eye troubles. Aqueous extracts of ripe fruits inhibit choline esterase activity of human plasma. (Chadha, 1972).

The fruits of *S. nigrum* contain glucose and fructose (15-20%), vitamin C and β-carotene. Green unripe fruits, however, contain glycoalkaloids and their eating is a toxic hazard to human beings as well as livestock. Ripe fruit contains very little alkaloids and can be consumed without ill effects. Seeds, forming 9.5% of

the weight of the fresh fruit, contain 17.5% protein on dry weight basic. They yield a greenish yellow oil. (Chadha, 1972).

Immature green fruits of the plant contain four steroidal glycoalkaloids viz. solamargine, solasonine, and α and β-solanigrin. All of them yield solasodine as the aglycone. It also contains a steroidal genin and tigogenin (m.p 206-07°). Solamargine and solasonine are present in leaves also. The total alkaloid content of fruits and leaves are 0.101 and 0.431% respectively. (Chadha, 1972).

Nematodes comprise a large and ubiquitous group of invertebrates which are highly diversified and have representatives in almost every kind of environment. They occur in unimaginable number and have a wide range of species, size and structures. A large number of nematode species are parasites of different kinds of plants and animals. Some species are macrophagus, others are saprophagus, and many species are herbivores or phytovores obtaining nourishment directly from plant tissues. It has been estimated that one acre of land can harbour as many as three billion nematodes. The growing awareness about phytoparasitic nematodes being limiting factor in agriculture productivity has led to a tremendous upswing in the

interest in a period of about six decades. Plant Nematology has emerged as a separate and independent branch of science.

The root-knot nematode, a common name collectively given to the species of *Meloidogyne* (Chitwood, 1949), causes the formation of knots (galls) on the roots of a wide variety of plants. Especially, it is of great concern in sub-temperate, sub-tropical and tropical regions, and is considered to be the number one nematode problem of agricultural crops in most developing nations. One or more, of about 90 presently known species and two subspecies, are serious problem on almost all the plants that account for the food, fibre or timber supply.,

Four most common species of the genus *Meloidogyne* are *M. incognita*, *M. javanica*, *M. hapla* and *M. arenaria*.. They attack various vegetables commonly grown in different parts of the world. The species *M. incognita* and *M. javanica* are more prevalent than *M. arenaria* and M. *hapla* whereas *M. incognita* is the most common species.

The root-knot nematodes are sedentary endoparasites of underground parts of the host plants. They complete their life cycle within 30 days. The second stage juvenile commonly known as larva, is the infective stage of *Meloidogyne*. It moves slowly in

the soil and develops into male if fails to come in contact with a host plant. The juveniles, usually, seem to be attracted towards the host roots. On coming in contact with the root they immediately penetrate into the inner tissues. Their preferential site of entry is the zone of elongation, close to the root tip meristem. The nematodes select either an epidermal cell or the site between the two adjacent cells whose cell walls are rather thin (Wyss *et. al,* 1992). The movement of juvenile within the roots in primarily intercellular (Nemec, 1910; Endo and Wergin, 1973), but intracellular migration has also been noted (Christie, 1936). Metabolical and cytological changes in undifferentiated cells of the vascular system usually occur after their entry. The affected cells enlarge abnormally to form "Giant Cells", which serve as the food tank for them. Parenchymatous cells adjacent to the giant cells become active and divide at a faster rate. This hyperplastic response leads to enlargement and eventually to gall formation in the affected part of the root. The giant cells support in the development of the young nematodes which increase considerably in width and gradually become flask-shaped. The entire root system,externally and internally,is being affected. The efficiency of vascular system to transport water, minerals and food decreases. It causes shortening of roots and reduction in top

growth, premature defoliation and wilting (especially in hot and dry areas). In crop fields the chlorotic patches of plants are the visible symptoms of root-knot disease.

Kostoff and Kendall (1930) advocated that the infection of root-knot nematode led to the formation of giant cells or syncytia, through the dissolution of the cell wall and the coalesecing of their contents in *Nicotiana*. Christie (1936), also supported the same process of syncytial formation, on root-knot infections in tomato roots. He observed highly enlarged undifferentiated cells with swollen nuclei near the head of the nematode. There was progressive dissolution of cell walls followed by the coalescence of protoplasm during the expansion of the syncytium. Similar mechanism of giant cell formation through cell wall dissolution have been proposed for a number of hosts infected by the species of *Meloidogyne* (Krusberg and Nielsen, 1958; Dropkin and Nelson, 1960; Owens and Specht, 1964; Littrell, 1966). On the contrary, in some other plants (Huang and Maggenti 1969; Paulson and Webster, 1970; Jones and Northcote, 1972; Jones and Dropkin, 1976; Jones and Payne, 1978) any evidence of cell wall dissolution or breakdown, during the formation of giant cells, was not noticed. The developing giant cells in the roots of *Vicia faba* were examined

by Jones and Payne (1978) under electron microscope. He did not find dissolution of cell walls and concluded that cell wall breakdown played no part in giant cell formation. The multinucleate condition arose, primarily, from repeated mitosis without cytokinesis.

In the giant cell, the nuclear aberrations ranged from a single hypertrophied nucleus to several small and large nuclei with varying stages of membrane deterioration and lobulated periphery. They were, generally, irregularly lobed with a tremendously increased surface area.

The cytoplasm of a young giant cell becomes dense as it carries golgi apparatus, mitochondria, ribosomes, polysomes and endoplasmic reticulum in abundance. (Jones and Northcote, 1972; Jones and Dropkin, 1976; Jones and Gunning, 1976; Jones and Payne, 1978; Wergin and Orion, 1981).

Galling is one of the earliest host response in root-knot nematode infections. Molliard (1900) observed galls on the root of melon, *Coleus*, *Begonia* and reported that after invasion the growth of primary and secondary roots may be arrested and lateral roots frequently developed near the site of invasion. The galls were induced on tomato roots by the larve of *Meloidogyne*

incognita without their actual entry into the root. (Schuster and Sullivan, 1960). They concluded that only the stylet penetrated into the dividing cells of the root secreted materials that stimulated host tissues to form galls.

Gall formation in *Gardenia spp.* infected with *M. incognita*, *M. incognita acrita* and *M. hapla* was studied by Davis and Jenkins (1960), who found that cortical and stelar proliferation accompanied all infections. *Meloidogyne javanica* infection on soyabean roots caused hypertrophy, hyperplasia and giant cell formation in the tissues surrounding the nematode head that consequently led to gall formation (Ibrahim and Massoud, 1974). According to Siddiqui and Taylor (1970) gall information is attributable to hypertrophy of the cortical tissues and egg mass production.

At infection sites of the roots of *Zingiber officinalis*, the cells of endodermis and pericyle were found suberized as well as liginified (Huang, 1966). Division of stele in galled roots of *Lycopersicon pimpinellifolium* infected with *M. incognita* was observed by Farooq (1973). Root-knot nematode stimulated the cells of pericycle to divide, forming parenchymatous outgrowths which grew into lateral roots. Sometimes the inner most

parenchymatous cells became differentiated into xylem elements of irregular shapes.

Siddiqui *et. al.*, (1974) observed the formation of xylem in the roots of *Lagenaria* infected with root-knot nematode. Pasha *et al.*, (1987) noted irregular scattering of vascular elements especially the vessels. Discontinuity of vascular tissues, significant reduction in vessel element dimensions, hypertrophy, hyperplasia, thickening of cell walls, granular cytoplasm, and enlarged nuclei and nucleoli were the most commonly occurring events in the tissue around the nematode head.

It is apparent that the root-knot nematode, once within the root, secretes a material that causes hypertrophy as well as hyperplasia. Owens and Specht (1964) suggested that hypertrophy of host tissues could be a response to mechanical pressure exerted by the enlarging nematode or to lateral movement of growth regulators around the cavity created by the developing nematode. The mechanical pressure concept proposed by Owens and Specht (1964) did not seem appropriate in explaining Schuster and Sullivan's (1960) concept of gall formation without the actual entry of the root-knot nematode. The alternate proposal by Owens and Specht (1964) suggested

that the role of growth regulator might be possible when one considers the generalized response of tissues to the nematodes.

Balasubramaniam and Rangaswami (1962) conducted an experiment in search of indole compounds from the root extracts of *Abelmoschus esculentus* infected with the root-knot nematode, *M. javanica*. When extracts of galled tissues and known samples of IAA and idole-butyric acid were chromatographed, the gall extracts were found to contain indole compounds which were thought to play a part in gall formation.

PROPOSED PLAN OF WORK

1. To study the effect of different inoculum levels of M. incognita on the development of giant cells in *Solanum nigrum roots*
2. To trace the different cytological changes leading to the formation of giant cells.
3. To study the damage caused by root-knot nematode infection to vascular tissues in *S. nigrum roots*
4. To trace the histopathological changes, in roots leading to gall formation.
5. To study the correlation between extent of damage caused to vascular elements and reduction in growth of plants and physiological symptoms resulting from disturbed physiology of infected plants.

Review of Literature

REVIEW OF LITERATURE

Root-knot nematodes *(Meloidogyne spp* Goeldi) Chitwood 1928 are the most important and the best known nematodes that cause root-knot disease in a wide variety of cultivated and wild plants. *Meloidogyne* spp. cause the formation of familiar knots or galls on the roots of susceptible host plants resulting in severe growth retardation of plants. They have evolved very specialized and complex host parasite relationship.

Root-knot nematodes are known to parasitize more than 2000 species of plants, but host parasite relationships have been investigated only in few plants (Webster, 1969, 1975; Taylor and Sasser, 1978; Hussey, 1985). The first record of any nematode injury of vegetables dates back to 1855 when Berkeley reported root-knot nematode damage to cucumber in an English green house. Root galling induced by *Meloidogyne* spp. is the well known host response which involves the production of abnormally large multinucleate, cells known as giant cells, in the vascular tissues of susceptible plants. Generally, root damage caused by parasitic nematodes is reflected, on the above ground portion of the plant, as poor shoot growth, leaf chlorosis and

even death of plants resulting in low yield and poor quality produce.

The stresses inflicted upon by the nematodes on the plants are manifested in the form of lesser tillering, yellowing and stunting of plants and finally low productivity. The above ground symptoms are similar to those associated with any root injury that result in reduced amounts of water uptake by plants. Flowering is scanty and fruits are either lacking or of poor quality (Jenkins and Taylor, 1967). *Meloidogyne* spp. also interfere with the process of nitrogen fixation in the nodules of leguminous plants.

The life cycle of *Meloidogyne* starts when second-stage juveniles infect roots of susceptible plants. They penetrate just at the root tips (Christie, 1936); or at the region of cell elongation just behind the root cap (Linford, 1939); or at the tips of young roots in the region of tissue differentiation; or any where from the root cap back to the region of root hair formation; or through the loose ruptured cells of enlarging tuberous roots (Krusberg and Nielsen, 1958); or in the region of cell differentiation and elongation. (Siddiqui and Taylor, 1970). When one of the juveniles punctures the cell wall and enters the root tissue, many juveniles follow it (Godfrey and Oliveira, 1932;

Linford, 1942; Peacock, 1959; Siddiqui, 1971; Hisamuddin, 1992; Youssef, 2001).

The nematodes select either an epidermal cell or the site between the two cells where the cell walls are thin (Wyss *et al.*, 1992). The movement of juveniles within the root is primarily intercellular (Nemec, 1910; Endo and Wergin, 1973) but intracellular migration has also been reported (Christie, 1936; Krusberg and Nielsen, 1958).

Wyss *et al.*, (1992) observed the parasitic behaviour of second-stage juveniles (J_2) of *Meloidogyne incognita* in roots of *Arabidopsis thaliana* with the aid of video-enhanced contrast light microscopy and time lapse techniques. The juveniles invaded the root, primarily in the region of elongation, close to the meristematic zone. Invasion was achieved, in most cases, by destroying epidermal and sub-epidermal cells, while intercellular invasion between epidermal cells was less frequent. Inside the root, the juveniles oriented themselves always in the direction of the root tip and migrated towards it between cortical and meristematic cells, without causing any damage. Occasionally attempts were made for at intracellular migration through cortical cells but failed due to insurmountable barriers. When the juveniles

reached the root apex, they turned around and migrated backward towards the differentiating vascular cylinder. Within the differentiated cylinder migration, eventually, was stopped and giant cell induction initiated.

The terms syncytium and giant cell were used interchangeably and considered as synonyms till recent past. The syncytium and the giant cell have been characterized on the basis of their mode of formation. Syncytium is a multinucleate transfer cell arising from extensive cell wall dissolution of the neighbouring cells. The multinucleate state arises by the preexisting nuclei of formely intact host cells. Giant cell is a multinucleate transfer cell in which the multinucleate condition results from multiple mitoses in absence of cytokinesis. Giant cell formation is not accompanied by wall dissolution. Giant cells are invariably formed by root-knot nematodes (Endo, 1987).

With the help of light microscope, Jones and Dropkin (1976) demonstrated the absence of cell wall breakdown in giant cells of soybean roots, infected with *Meloidogyne incognita*. Jones and Payne (1978) examined early stages of nematode-induced giant cell formation, under electron microscope, in the roots of *Impatiens balsamina* infected with *M. incognita* and *M.*

javanica. Nuclear divisions were detected 24 h after inoculation in the cells upto two layers from the head of the nematode. These divisions were considered to be the first sign of giant cell formation. After 48 h, two, four and eight nuclei were observed in the cells near the nematode head. In early stages of giant cell formation, cell wall breakdown and cell fusion were not evidenced under electron microscope. Wall gaps or holes in a continuous cell wall were also not detected. The cell wall of a giant cell, however, consisted of thick and thin areas.

The nuclei, in the giant cells of root-knot nematode infected sweet potato roots, varied in size, shape and other characteristics. They were sometimes approximately 100 times larger than the nuclei of the neighbouring cells. Their shapes were spherical, elongate, pyriform or dumb bell like, and sometimes possessed projections (Krusberg and Nielsen, 1958; Mohali 1999). Owens and Specht (1964) observed nuclear changes ranging from a nucleus exhibiting a hypertrophied nucleolus with the apparent absence of a nuclear membrane to nuclei with various stages of membrane deterioration and a lobulated periphery. Nucleolar fragmentation resulted into small granules which stained like nucleoli and remained scattered throughout the nucleus.

In *Solanum melongena,* infected with *M. incognita*, Pasha *et al.*, (1987) observed feeding site of nematode mainly in stelar region. The cells in the feeding site exhibited hypertrophy, hyperplasia, thickening of cell wall, granular cytoplasm and enlarged nuclei and nucleoli. Abnormal xylem, formed in response to infection, occurred in irregular patches causing discontinuity in vascular tissue. In banana roots, Bilquees and Jabeen (1994), observed cavities that originated by cell proliferation, cell wall destruction and lysis of cells in response to the pressure exerted and toxic materials secreted by *Meloidogyne* species.

While observing penetration, development and migration of *M. arenaria* in the roots of three *Myrobalan plum* clones genetically characterized for their resistance to root-knot nematodes, Voisin *et. al.*, (1999) suggested that the resistance phenomenon did not act on the very early nematode penetration but acted later on by preventing feeding site induction and development into the third stage. There was no significant difference in the number of emigrated juveniles between the resistant and susceptible clones.

Wiggers, *et. al*, (2002) found that giant cells induced by *Meloidogyne* species were characteristically enlarged and multinucleate. Each nucleus had more chromosomes than unaffected root tip cells. In *Vicia faba,* only euploid nuclei were detected whereas in *P. sativum* euploid and aneuploid nuclei were reported. The origin of the increased chromosome number is not known. The presence of strictly euploid nuclei in *V. faba* suggested endomitosis and possibly nuclear fusion as was reported by Huang and Maggenti (1969). Individual nuclei within a single giant cell also had greatly increased DNA contents, ranging upto 16 times that of unaffected cells.

Despite these well known nuclear abnormalities of giant cells, little is known about the relationship between multinucleate condition of the giant cells and development of the nematode parasite. Histochemical alterations in severely infected plants comprised of enhancement in DNA, RNA, total protein and ascorbic acid contents, when compared with healthy plants (Sharma and Trivedi, 1998; Sharma and Mathur, 1999).

Severity in disease symptoms, anatomical anomalies and in chemical imbalances were found to be influenced by the amount of inoculum. Reduction in weight, in length and diameter of

roots; enhancement in galling, hairiness and other malformations in roots; and increase in nematode population with an increase in initial inoculum level were noticed by Park *et al.*, (1999). Lettuce was found to be more severely affected by Race 2 than Race 1 of *Meloidogyne incognita* (Kryzanowski *et al.*, 2000). Higher inoculum levels of *M. incognita* produced maximum number of galls and resulted in significant reduction in growth of *Allium porrum L.* (Rombati and Dhanachand, 2000). They observed higher protein and lipid contents in plant shoot at higher inoculum level, and also found a negative correlation between nematode multiplication and inoculum level. The number of storage roots in cassava decreased when plants were inoculated with *M. incognita* even after 88 days after plantation (Makumbi *et al.*, 2000). Out of 57 species of different plants tested for pathogenecity, *Solanum nigrum* was reported to be invaded by lesser number of juveniles but after penetration their development was normal (Ehwaeti *et al.*, 1999).

Materials & Methods

MATERIALS AND METHODS

The different material to be used and methods to be employed during the course of proposed experimental programme have been generalized as follows:

Test Plant and Pathogens:

In the proposed plan of work Black Nightshade *(Solanum nigrum L.)* will be selected as the test plant, and the root-knot nematode (*Meloidogyne incognita,* Kofoid and White) Chitwood as the test pathogen.

Raising of Test Plant:

The seeds of the test plant, *Solanum nigrum* will be surface sterilized with 0.5% sodium hypochlorite (NaOCl) for five minutes and thoroughly washed five times with distilled water. The seeds will then be sown in autoclaved clay pots of diameter 15 cm; containing steam sterilized soil and manure in fixed proportion of 2:1. One week old seedlings will be used for inoculation.

Collection of Nematode Inoculum *(Meloidogyne incognita)*

Roots of brinjal *(Solanum melongena)* infected with root-knot nematode will be collected from brinjal field. Root-knot

nematode species will be identified on the basis of the characteristic perineal patterns.

Pure Culturing and Maintenance of Inoculum:

A single egg mass of *Meloidogyne incognita* will be surface sterilized in 1:500 solution of chlorox (calcium hypochlorite) for five minutes and washed thrice in sterilized distilled water. The egg mass will be allowed to hatch in sterilized water at 37°C. Eggplant seedlings, raised in 25 cm pots containing autoclaved soil, will be inoculated with the second-stage juveniles (J_2) thus obtained. After about five months, soil of inoculated pots will be examined to ascertain the establishement of nematodes. The subculturing will be done by inoculating new egg plants with at least 15 egg masses obtained from pure culture in order to maintain sufficient inoculum throughout the course of investigation.

Isolation and preparation of Nematode Inoculum:

The egg masses of *M. incognita* will be removed from the infected plants and allowed to hatch at 27°C in an incubator. After every 24 hour the nematode suspension will be collected in beakers. The number of second-stage juveniles of *M. incognita* will be estimated with the help of counting dish under the

stereoscopic microscope. An average of three to five counts will be made to determine the population of nematode juveniles in the suspension.

Inoculation with Nematode:

After one week of seedling emergence, holes of 5-7 cm depth around the plants, within a radius of 2cm from the plant, will be made in which a counted number of second stage juveniles will be transferred with the help of sterilized pipette. The holes will then be plugged with sterilized soil. Regular watering will be done to maintain the soil moisture till the termination of the experiment.

Histopathological Studies:

Histopathological studies of *Meloidogyne* species require the capacity to prepare thin sections of infected root tissue with a minimum distortion. Samples of healthy and infected root tissues will be taken in all histopathological studies. Roots will be dug, not pulled, out of the soil and washed gently, but thoroughly, under running tap water to remove all soil particles. Tissues will then be cut into smaller pieces and placed in a container of fixative as soon as possible. Small roots will be cut with a sharp

razor blade into one cm long fragments while immersed in a drop of water.

Fixation: Fixation kills and hardens the tissue and preserves the cellular structure. One of the best and common fixatives is formalin-aceto-alcohol (FAA): 90ml of 50% ethanol, 5ml of glacial acetic acid, and 5ml of 37% formaldehyde. It will be used for fixing root tissues.

In fixation, the tissues will be submerged in a volume of fixative at least 10 times greater than that of the volume of the tissue to ensure that the fixative did not become over diluted by water of the tissue. Tissue will be kept embedded in the fixative for a minimum of 24 hrs to several days, depending on its thickness. Materials will be stored in the fixative.

Dehydration: The dehydration is accomplished by moving the tissue stepwise through increasingly higher concentrations of alcohols. Tertiary butyl alcohol (TAB) dehydration schedule (Johansen, 1940) will be followed (Table 1) for dehydration. Care will be taken that the material did not dessicate. The time, the material is allowed to remain in the various dehydrating solutions, depends on its thickness.

Infiltration: The alcohols in the tissues are replaced by paraffin so that the tissue gets saturated with a pure solution of paraffin. From the tissue TBA solution will first be replaced with 1:1 mixture of 100% TBA and paraffin oil. The tissue will be allowed to remain in this solution for 1 hour or more, depending on its thickness. The tissue in the TBA-paraffin oil mixture will then be placed on top of the solidified paraffin and will be covered with a layer of the TBA-paraffin oil solution. This container will be placed uncovered in an incubator set at a temperature slightly above the melting point of the paraffin. The tissue will sink into the bottom of the container as the paraffin melts. After one to three hour, the TBA and paraffin oil mixture will be poured off and be replaced with pure melted paraffin. The uncovered container, then, will be placed back in incubator for about three hours. This step will be repeated at least once more. The tissue embedded in paraffin will be left overnight in incubator. After one more infiltration with fresh melted paraffin the tissue will be ready for embedding.

Embedding: In the process of embedding, the tissue will be positioned in cooling paraffin so that it could be sectioned after hardening. Molds for embedding will be prepared in the laboratory from folded paper. Molds will be coated with a thin

layer of glycerin. The tissue sample will then be placed carefully into the mold with heated forceps and additional melted paraffin will be added to fill the mold. This step will be done on a hot plate set at 60°C. The filled mold will be next moved to a cool surface on the laboratory bench. As soon as the paraffin would begin to solidify on the bottom of the mold, the tissue will be rapidly oriented in the desired fashion with a heated dissecting needle. Once the paraffin begins to solidify over the top of the mold, the mold will be plunged into ice water. After hardening, extra paraffin will be removed from the mold and be cut into smaller blocks which could either be mounted on wooden blocks with melted paraffin or inserted directly into the microtome.

Sectioning: Sectioning of paraffin blocks will be done on a rotatry microtome equipped with a sharp knife. Excess paraffin surrounding the tissue will be trimmed away before sectioning, leaving at least one mm around the tissue. Thin sections of about 8-12μm in thickness will be cut for histological studies. The sections will be cut in the form of ribbon which will be mounted immediately on the slides.

Ribbon Mounting: Ribbons will be cut into shorter lengths so that they could get fit into the slides. The surface of slides will

be coated with a small amount of Haupt's adhesive which consists of one gram powdered high grade gelatin, 100ml distilled water, two gram phenol crystals, and 15ml glycerin. Before the adhesive dries up the slides will be flooded with a 2-3% formalin solution. The slides will be placed on a warming tray held at 35 to 40°C, and segments of the ribbon will be floated on the slides. As the slide will warm up, the ribbon will flatten out and the liquid will evaporate. After several hours, when the slides will become completely dry, they will be stored indefinitely.

Staining: For staining the tissue, Sass (1951) schedule will be followed, (Table-2). After completion of the staining procedure the mounting medium will be applied to the surface of the slide before the xylene evaporates, and a cover slip will be lowered down gradually over the slide. Finished slides will be left flat to dry for at least 24 hours, at room temperature. However, the medium will harden better if the slides were held on a 60°C warming tray overnight. The slides will be examined under the stercoscopic microscope. Necessary photographs will be taken.

Table 1: Tertiary Butyl Alcohol Dehydration Schedule: (TBA)

Step	(%) Alcohol	Time	Quantity (ml) Needed for solution			
			Distilled Water	95% Ethanol	100% Ethanol	100% TBA
1	50	2 hr or more	50	400	0	10
2	70	Overnight	30	50	0	20
3	85	102 hr	15	50	0	35
4	95	1-2 hr	0	0	25	75
5	100	1-3 hr	0	0	0	100
6	100	1-3 hr	0	0	0	100
7	100	1-3 hr	0	0	0	100
8	100	Overnight	0	0		

Table 2: Safranin and Fast Green Stain:

Step	Solution	Time
1.	Xylene	5 min.
2.	Absolute ethanol	4 min.
3.	95% ethanol	5 min.
4.	70% ethanol	5 min.
5.	50% ethanol	5 min.
6.	30% ethanol	
7.	1% aqueous safranin	1-12 hr.
8.	Rinse in tap water	
9.	30% ethanol	3 min.
10.	50% ethanol	3 min.
11.	70% ethanol	3 min
12.	95% ethanol	3 min.
13.	0.1% fast green FCF in 95% ethanol	5-3 sec.
14.	Absolute ethanol	15 sec.
15.	Absolute ethanol	3 min.
16.	Xylene-absolute ethanol (1:1)	5 min.
17.	Xylene	5 min.
18.	Xylene	5 min. or longer

Experiment-I

EXPERIMENT NO. 1:

EFFECT OF DIFFERENT INOCULUM LEVELS OF *Meloidogyne incognita* on *Solanum nigrum.*

Low or high population densities of *Meloidogyne incognita* produce different effects on plants. At low inoculum levels sometimes the plant growth is stimulated, but at other times it is suppressed. Wallace (1971) reported increased plant growth at lower population densities and decreased growth at higher densities Dropkin (1954) inoculated tomato roots with a single juvenile of *M. incognita acrita* and measured the size of the gall so produced. He hypothesized that each individual nematode produced a finite response on the root tissue. Thus by measuring the gall size, the number of nematodes present the gall could be predicted. In heavily infested roots this hypothesis did not work.

The following work was carried out to determine the effects of different inoculum levels of *M. incognita* on (i) length of plant, (ii) weight of plant, (iii) number of flower and number of fruits.

MATERIALS AND METHODS

Preparation of Test Plants:

Three week old seedlings of *Solanum nigrum* were transferred to 30 cm diameter clay pots filled with steam sterilized

soil (clay: sand: manure : 7 : 3:1). After one week the seedlings were thinned to one seedling per pot.

Inoculation:

Egg masses of *M. inconita* were collected from roots of egg plants maintained in glass house for pure culturing. The egg masses were incubated in coarse sieves (3 inch diameter) fitted with double layered tissue paper and placed on a Baermann funnel containing sufficient amount of water. After 72 h the juveniles were collected and stored in a beaker at 7°C. The number of juveniles were counted and standardized. Different inoculum levels comprising of 05, 50, 500, 5000 juveniles per 10 ml of water were used to inoculate one week old seedlings. Nematode suspension, containing desired inoculum levels, was added into the pots with the help of sterilized pipettle. Within a radius of two cm from the plant, three to four cm deep holes were made through which suspension was introduced. The holes were plugged with soil. Soon after inoculation, each treatment was replicated five times and the pots were arranged in randomized block design. Uninoculated plants served as control. The plants were watered regularly when required and were harvested 45 days after inoculation.

Plant Growth:

After termination of the experiment, lengths, fresh weight, dry weights of root and shoot, number of flower and fruits of inoculated and uninoculated plants were determined. Root and shoot length was measured with the help of meter scale. Roots and shoots of plants of each treatment were weighed when fresh, and then kept in bamboo paper envelopes. The envelopes were left in an oven for 48 hrs at 80°C, and then weighed to obtain their dry weights. The number of flowers and fruits was counted visually.

RESULTS AND DISCUSSION:

Root and Shoot Lengths:

Initial inoculum levels (P_i=05J_2, 50J_2, 500J_2, and 5000J_2) of *Meloidogyne incognita* exhibited different effects on lengths of roots and shoots, and fresh and dry weights of roots and shoots of the plant (*Solanum nigrum).* Their impacts were more pronounced on roots than on shoots when compared with uninoculated (control) plants. In comparison to control, root and shoot lengths remained unaffected at P_i=05J_2. With an increase in inoculum density to 50 juveniles per plant, an increase in length of both roots and shoots was observed. The increase, however, was

non significant. At higher inoculum level (P_i=500J_2), root and shoot lengths decreased significantly (P=0.005). At the highest inoculum level, the lengths were drastically reduced when compared with the length of control plants and with the lengths of plants at least of the inoculum levels. Reductions were higher in root lengths than in shoot lengths at P_i=500J_2 and at P_i=5,000J_2.

Root and Shoot Weights: Fresh weights of roots as well as shoots remained unaffected at lowest (Pi=05J_2) inoculum level. Both root and shoot fresh weights increased, though non-significantly, at the initial inoculum level of Pi=50J_2 per pot. Maximum reduction in fresh weight was observed at the highest inoculum level (Pi=5000J_2). The reduction in root and shoot weights at highest inoculums level was significantly higher in companion to the weights of control plants and the plants at other initial inoculum levels. The dry weight of roots and shoots neither increased nor decreased at P_i=05J_2. Similarly, in dry weights, the fresh of increase or decrease were similar to fresh weights. Root and shoot dry weights non-significantly increased at p_i=50J_2 and significantly decreased at p_i=500J_2 and pi=5000J_2 respectively, (Table 3).

While comparing nematode inoculated plants with each other, it was found that at higher (P_i=500J_2) inoculum level root and shoot weights were significantly (P=0.05) lower than the weights at P_i=05J_2 and P_i=50J_2 per pot, and higher than at P_i=500J_2. At highest inoculum level (P_i=5000J_2), root and shoot weights were significantly (P=0.01) lower as compared with the weights at P_i=05J_2 and 50J_2 and at P_i=500J_2 (Table 3).

Number of Flowers:

The number of flowers per plant decreased with an increase in the initial inoculum level. The number of flowers increased non-significantly at initial inoculum level of P_i=05J_2 when compared with control. A non-significant decreased in the number of flowers per plant observed at P_i=50J_2. The number of flowers significantly (P=0.01) decreased at the initial inoculum level of P_i=500J_2. A significant (P=0.01) decrease in the number of flowers was observed at an initial inoculum level of P_i=5,000J_2 (Table 3).

Number of Fruits:

The number of fruits per plant in comparison to control, increased non-significantly at the initial inoculum level of P_i=05J_2. A non-significant decrease in the number of fruits was observed at P_i=50J_2, when compared with control plants. In comparison to

control, a significant (P=0.01) decrease was noticed at an initial inoculum level of P_i=500J_2 and P_i=5,000J_2 (Table 3).

Plant response towards *Meloidogyne* could be reflected by the symptoms developed on the roots and the shoots. Alteration in root anatomy are peculiar characteristics to this specific type of host-parasite relationship. The most striking above ground symptom of root tissue disease is the stunting of the affected plant. However, it is not necessary that the plant would always develop the characteristic symptoms. It is evident from our studies that if primary inoculum comprised of a very low amount, it did not produce above ground symptoms. Although, juveniles entered the roots and caused gall formation but their overall effect on the plant growth was negligible. From our findings it might be concluded that low population density of second stage juveniles induced infections without causing any significant damage to the plant. Earlier workers like Tylor (1933), Dropkin (1954), and Dropkin and Boone (1966) used single larval inoculation to find its response at the site of infection. They did not mention its affect on the growth of the plant.

At both higher inoculum levels (P_i=500 and P_i=5000J_2) growth of the plant suppressed significantly. The damage to the

plant, by *Meloidogyne* species, involves several mechanisms. The plant growth might be affected due to removal of nutrients by the nematode. The nutrient transport from the root towards shoot is hampered due to anatomical abnormalities in the galled regions. Moreover, photosynthates are diverted towards the giant cells that act as metabolic sinks for the organic compounds. All these malfunctions contribute in suppressing plant growth and yield (Hussey, 1985). Root-knot nematode infection markedely retards the rate of absorption by the roots and also affects the rate of translocation towards the shoot apices.

Increase in amount of initial inoculum level of *M. incognita* was responsible for increased loss in fresh and dry weights of *Solanum nigrum*. Bird (1962, 1968) opined that the rate of photosynthesis in fomato decreased with the increase in initial inoculum level.

In this experiment, it was found that the plant growth characters like root and shoot length, their fresh and dry weights, of the *M. incognita* infected were adversely affected as the levels of inoculum increased from 500 to $5000J_2$ per plant. The reduction was significant at the highest inoculum level (P_i=5,000J_2) when compared with control plants. Adverse affects

on plant growth with the increase in primary inoculum levels of *Meloidogyne* species have been reported by several workers (Christie, 1936; Krusberg and Nielsen 1958; Wallace 1969; Ferris 1974; Barker and Olthof 1976; Nordacci and Barker 1979; Kinloch 1980, 1982; Rodriguez-Kabana and Williams 1981; Appel and Lewis 1984; Ibrahim and Lewis 1985; Fazal *et al*, 1996). Seinhorst (1965) observed a measurable damage occurring only when the nematode population density exceeds a certain limit. In the present study the growth parameters and nematode inoculum density showed positive linear relationship. Wallace (1963) and Oostenbrink (1966) opined that the increase in nematode populations and subsequent reductions in the yield of crops or other manifestations of pathogenic effects are directly influenced by initial density of the nematode in soil.

Table 3: EFFECT OF DIFFERENT INOCULUM LEVELS OF *M. incognita* ON GROWTH AND YIELD OF *S. Nigrum*

	Length (cm)		Fresh Weight (g)		Dry Weight (g)		Total number of	
	Root	Shoot	Root	Shoot	Root	Shoot	Flowers	Fruits
Control	28.9	65.7	2.64	28.56	1.27	9.54	42.4	32.6
5J2	29.8	66.5	2.58	28.44	1.23	9.46	40.7	28.7
50J2	24.3	59.4	2.54	24.81	1.09	8.52	32.8	23.2
500J2	18.6	47.4	2.21	19.78	0.92	5.12	21.6	10.6
5000J2	11.3	27.2	1.77	12.44	0.72	2.68	9.5	3.8
LSD P=0.05	5.3	12.4	0.32	7.89	0.23	1.54	9.12	7.54
LSD P=0.01	7.4	15.7	0.63	9.72	0.61	1.91	12.21	10.13

N.B: Each value is a mean of five replicates

Experiment-II

EXPERIMENT NO. 2

EFFECT OF FLY ASH WATER EXTRACTS ON PLANT MORPHOLOGY, BIOMASS PRODUCTION AND YIELD OF *Solanum nigrum.*

Solanum nigrum is a wild plant belonging to the family solanaceae, commonly called as Black Nightshade. It is an important medicinal plant distributed throughout the India and Pakistan. Decoctions of the plants, after transient stimulation, depress the central nervous system and the reflexes of the spinal cord. Extracts of the plant affect the rate and amplitude of respiration (Chadha, 1972). The juice of fresh leaves is reported to produce dilatation of the pupil. Berries are considered to possess tonic, diuretic and cathartic properties.

Air pollution is increasing tremendously due to industrial growth and other activities including power generation plants and transportation. The emission of gaseous or particulate nature are causing damage to the plant morphology, anatomy, physiology and even to medicinally active components of the cells. The thermal power plant, Kasimpur (Aligarh) was selected as the pollution source for this study. The present study was carried out

in order to find out the effect of water extracts of fly ash on plant morphology, biomass production and yield of *Solanum nigrum*.

MATERIALS AND METHODS:

One week old seedlings were transferred to the autoclaved clay pots, containing steam sterilized soil and manure in the ratio of 3:1. The fly ash water extracts were prepared by keeping distilled water for 24 h in 100gm of fly Ash: as summarized below:

C – control

T_1 – Extract of 100gm fly ash with 200ml of DW.

T_2 – Extract of 100gm fly ash with 300 ml of DW.

T_3 - Extract of 100gm fly ash with 400 ml of DW.

T_4 - Extract of 100gm fly ash with 500 ml of DW.

All the plants were watered with fly ash extracts. The plants grown in untreated water served as control. Each treatment consisted of five replicates. Watering was done regularly and after two months, the crop was terminated. Soon after harvesting, length of plant and fresh weight were measured. The number of flowers and fruits were counted during the growing phase. Dry

weights were obtained after drying root and shoot parts separately.

RESULTS AND DISCUSSION:

The data revealed that the plant length decreased significantly in the treatments T_1 and T_2 and non-significantly in T_3 and T_4. Reduction was higher in T_1 plants when compared with control, significant reduction ($P=0.01$) in T_1 and ($P=0.05$) in T_2 plants were observed. In T_3 and T_4 plants, reductions in plant length were noticed in the roots as well as the shoots. However, the differences were non-significant. From this observations it is obvious that, higher concentrations of fly ash extract were not favouring normal plant growth. This unfavourable response of the plants towards the fly ash extracts might be due to change in pH of the soil. Effects of fly ash amended soils have been work out by several workers. Some authors are of the opinion that fly ash adds micro and macro nutrients to the soil which enchances plant growth. (Druzina *et al*, 1983) enhancement in growth was attributed to change in soil pH, water holding capacity, porosity and ion exchange capacity. (Jones and Straughan 1978; Adriano *et al*, 1980; Elseewi *et al.*, 1981). Others have proposed that fly ash addition into the soil caused growth retardation because of

amplification of heavy metal elements viz. zinc, copper, magnesium cobalt etc. (Beasley 1942; Eveling, 1969; Eller 1977; Fluckinger *et al.* 1978).

Fresh weights of the roots and the shoots and also the dry weights of roots and the shoots decreased significantly in the soil containing higher concentrations of fly ash extracts. Significant reductions were observed in both T_1 ($P=0.01$) and T_2 ($P=0.05$) as compared to control. The plants of the treatments T_3 and T_4 did not exhibit any significant change, in root weight and shoot weight when compared with control. This aspect of observation also strengthen the view that high concentration of fly ash had injurious effects on the plants. These effects might be due to some metabolic reactions as no visible injury symptoms were noticed on the roots and the shoots.

The yield of the plant in terms of number of flowers and number of fruits exhibited reduction in the treatments T_1, T_2 and T_3; when the comparisons were made with control. The plants of the treatments T_1 did not produce any flower and eventually the fruits throughout the season. Sparse flowering was noticed on T_2 plants, which did not bear any fruit. Scanty flowering and fruiting were seen in T_3 plants. There was a significant ($P=0.01$)

reduction in flowering and fruiting on T_3 plants when compared with control. Flowering and fruiting non-significantly decreased on T_4 plants.

After going through the literature it is apparent that no work has been done with fly ash extract. Higher concentrations of fly ash extracts have been reported to be beneficial by some workers and toxic by other worker. Growth and yield was reported to be increased at higher concentrations in some plants (Druzina *et al.*, 1983). Retardation in plant growth and reduction in yield was enhanced at higher fly ash extract concentrations (Hodgson and Holliday, 1966; Ricks and Williams, 1974; Fluckinger *et al.*, 1978). The growth of *solanum nigrum* was retarded at higher fly ash extract concentrations. These concentrations also influenced on the production of biomass and formation of flowers and fruits. These effects were developed probably because of change in pH of the soil and due to addition of some water soluble heavy metal elements.

TABLE 4: EFFECT OF FLY ASH WATER EXTRACTS ON PLANT MORPHOLOGY, BIOMASS PRODUCTS AND YIELD OF *S. nigrum*

	Length (cm)		Fresh Weight (g)		Dry Weight (g)		Total number of	
	Root	Shoot	Root	Shoot	Root	Shoot	Flowers	Fruits
Control	28.8	63.4	2.34	27.08	1.12	8.44	43.9	31.6
T_1	19.6	45.5	1.45	20.61	0.83	5.79	--	--
T_2	23.2	52.8	1.73	22.47	0.96	6.98	2.3	--
T_3	26.3	57.1	2.17	24.83	1.02	7.88	20.2	9.6
T_4	27.8	58.9	2.26	26.25	1.09	8.12	40.3	27.6
LSD P=0.05	6.3	13.9	0.21	3.64	0.27	1.36	6.8	5.3
LSD P=0.01	8.7	16.4	0.56	6.19	0.62	1.87	8.7	7.1

N.B: Each value is a mean of five replicates

References

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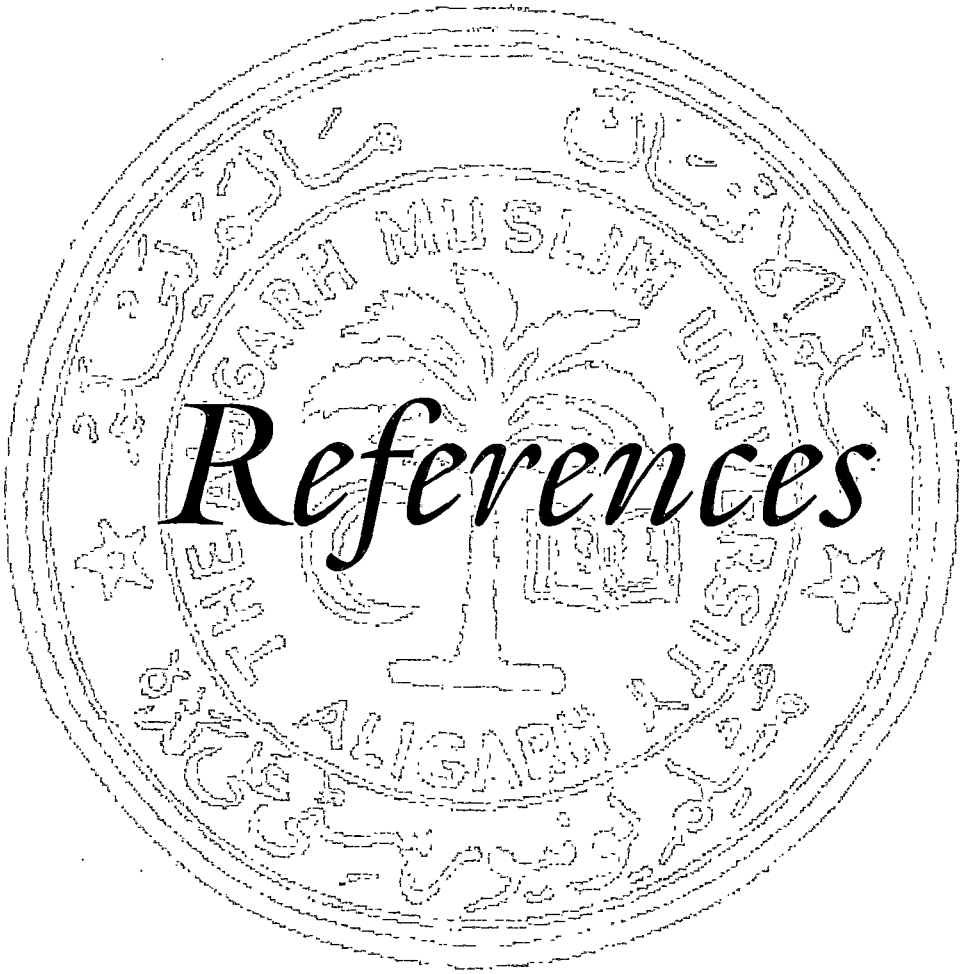
T_1	19.6	45.5	1.45	20.61	0.83	5.79	--	--
T_2	23.2	52.8	1.73	22.47	0.96	6.98	2.3	--
T_3	26.3	57.1	2.17	24.83	1.02	7.88	20.2	9.6
T_4	27.8	58.9	2.26	26.25	1.09	8.12	40.3	27.6
LSD P=0.05	6.3	13.9	0.21	3.64	0.27	1.36	6.8	5.3
LSD P=0.01	8.7	16.4	0.56	6.19	0.62	1.87	8.7	7.1

N.B: Each value is a mean of five replicates



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